REMARKS/ARGUMENTS

Claims 1-6, 8-10, and 14 have been examined and their rejections are addressed below.

Claims 2 and 9 have been revised to make more explicit an inherent feature of each of the claims. Applicants do not believe, or intend, that any narrowing or change in claim scope has occurred. Support for the revision to claim 2 is found at least in the instant specification at page 4, lines 15-25, and page 18, lines 29-32. Support for the revision to claim 9 is provided by the claim as previously presented.

No new matter has been introduced, and entry of the revised claims is respectfully requested.

Information Disclosure Statement (IDS)

Applicants acknowledge the assertion that the references listed on pages 67-71 of the specification are "not a proper information disclosure statement" under 37 C.F.R. § 1.98(b). Applicants also understand the indication that those references have not been considered by the Examiner unless cited on a form PTO-892 or as initialed on a form PTO-1449.

Applicants respectfully submit, however, that IDS documents filed April 1 and 29, 2005 are proper as indicated by the initialed copies returned with the Office Action mailed May 30, 2006. Moreover, Applicants believe that inclusion of the reference list on pages 67-71 is not improper and so believe that it may be retained.

Specification

The Office Action mailed May 30, 2006 includes a requirement for a new title "that is indicative of the invention to which the claims are directed". Applicants respectfully request that this requirement be held in abeyance pending determination of the final language of the claims during prosecution.

The Office Action mailed May 30, 2006 also objects to the inclusion of certain internet related text on pages 14, 21, and 22 of the specification. The relevant paragraphs on pages 14, 21 and 22 of the specification have been revised as presented above to remove embedded hyperlinks and to render them non-executable. No new matter has been introduced, and entry of the revised paragraphs is respectfully requested.

Drawings

The Office Action mailed May 30, 2006 includes objections to the drawings. With respect to Figure 22, the relevant paragraph on page 37 has been revised as presented above.

With respect to Figures 16, 17b, 17c, 22a, 22b, and 24-27, Applicants submit herewith seven (7) replacement drawing sheets containing Figures 16, 17a, 17b, 17c, 22a, 22b, and 24-27. These replacement sheets are believed to provide better images of Figures 16, 17b, 17c, 22a, 22b, and 24-27 as requested in the Office Action mailed May 30, 2006. No new matter has been introduced, and entry of the replacement drawings is respectfully requested.

Issue under 35 U.S.C. §112, First Paragraph

Claims 1-6, 8-10, and 14 were rejected under 35 U.S.C. §112, first paragraph as allegedly not enabled such that a skilled artisan could make and/or use the invention beyond the scope of "mouse embryonic stem (mES) cells or human embryonic carcinoma (hEC) cells". Applicants have carefully reviewed the statement of the rejection and respectfully traverse because no *prima facie* case of a lack of enablement has been presented.

As an initial matter, Applicants point out that the standard to be applied in establishing a *prima facie* case of non-enablement is set out in part at MPEP 2164.04, including the guidance provided by *In re Marzocchi*[1] and the other cases cited therein. With reference to *Marzocchi*, the standard states in part that

> "A specification disclosure which contains a teaching of the manner and process of making and using an invention in terms which correspond in

[1] 439 F.2d 220, 169 USPQ 367 (CCPA 1971).

> scope to those used in describing and defining the subject matter sought to be patented must be taken as being in compliance with the enablement requirement of 35 U.S.C. § 112, first paragraph, unless there is a reason to doubt the *objective* truth of the statements contained therein which must be relied on for enabling support." (underlining and italics added)

The above standard requires *objective* reasons to doubt the presumption of an enabling disclosure. Mere reliance on assertions of possibilities or conjectures are not enough. There must be objective reasons why undue experimentation is necessary to make and use the claimed invention.

Moreover, undue experimentation is not the same as the absence of experimentation. To the contrary, routine and repetitive experimentation, like that involved in the case of *In re Wands*[2], is entirely contrary to an assertion of non-enablement.

In the instant application, the disclosure, and thus claims, were enabling as originally filed for the full scope of the claims because no adequate and objective reasons were presented to doubt the ability to detecting the differentiation status of stem cells by detecting the expression of 5T4 antigen in said stem cells.

The instant rejection is based on a number of allegations presented in the Office Action mailed May 30, 2006 as follows

1) in the paragraph bridging pages 8 and 9, lack of demonstration "that 5T4 antigen is a definitive marker of differentiation or an indicator of pluripotency";

2) in the paragraph bridging pages 9 and 10, "there are no marker that can accurately assess differentiation status and pluripotency potential of ES cells" as reflected on page 2, lines 28-29 of the instant application; and

3) in the first full paragraph on page 10, "[t]he specification only provides guidance for mES and hEC cells, therefore it only enables mES and hEC cells."

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2 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988).

With respect to allegation 1), the statement of the instant rejection alleges that "incorporation of mES cell is not a measure of differentiation" and that although formation of chimeras "suggests that stem cells that to some degree are less differentiated were present, it does not determine the level of differentiation nor does it demonstrate its contribution to the animal." See page 8 of the Office Action mailed May 30, 2006.

The statement of the rejection then continues by speculating that "[i]t is possible that some of the cells incorporate into the animal but do not divide therefore not contributing to the development of the animal." The instant application's data regarding the incorporation of 5T4 antigen negative mES cells into chimeras is then construed as supporting the speculation. For example, the observation of 52% incorporation, turned into a focus on 48% non-incorporation, is used as supporting the allegation that some 5T4 antigen negative cells incorporated into a chimera do not develop because they were not pluripotent.

Applicants respectfully, but strongly, disagree because the examiner has apparently misunderstood the nature of the experiment and its possible interpretation.

mES cells are known to be able to contribute to functional germ cells in chimeras, and ES cell descendants are known to be represented among all cell types in resulting chimeric offspring. For example, a common technique is to introduce specific genetic changes into a mouse germ line through the use of ES cell chimeras (see Ramirez Solis R et al., 1993, Methods Enzymol. 225:855-878 as a non-limiting example).

The experiments described in the instant application indicate that the proportion of chimeric animals generated with different sorted populations of SSEA-1 positive mES cells stratified based on the presence or absence of 5T4. Therefore, it is inaccurate to interpret the results, where 48% of the 5T4 negative cell population did not incorporate into chimeras, as demonstrating that 5T4 negative cells are not pluripotent. The experimental method used involved use of a population of cells and was not a clonal evaluation or a clonal experiment. The experimental method for chimera generation is inefficient, and the efficiency of making chimeras is known to depend on various factors. So contrary to the additional assertion that "even a population determined 5T4 antigen negative mES cells is still a heterogeneous population of

mES cells that still have different differentiation and pluripotency potentials", the lack of 100% incorporation is not necessarily indicative of non-pluripotent cells.

Instead, the disclosed experiment is directed to the relationship between the absence of 5T4 expression and pluripotency compared to the presence of 5T4 expression and the lack of pluripotency. Using delivery of the different populations of cells as a non-clonal evaluation, the experiment showed that an approximately seven (7) fold increase in efficiency in forming chimeras using an SSEA-1+/5T4- cell population compared to an SSEA-1+/5T4+ cell population (see Figure 18 and page 60, lines 14-29). SSEA-1 is a well-known and validated indicator of pluripotency and the results disclosed here illustrate that the absence of 5T4 is a more sensitive indicator of pluripotency as it results in a greater yield in chimeras.

Therefore, and contrary to the view expressed in the statement of the rejection, the absence of 5T4 expression is an indicator of pluripotency.

With respect to allegation 2), Applicants respectfully point out that page 2, lines 28-29 state as follows: "there is no marker that can accurately assess both the undifferentiated integrity and differentiated state of stem cells". This is in contrast to the assertion in allegation 2) of no marker that can accurately assess the differentiation status and pluripotency potential of ES cells.

Applicants have not asserted the latter, which is in contrast to knowledge of markers which are useful and have been widely applied in research within the stem cell field. Some of these markers are discussed on page 2, lines 11-26, of the instant application. But as noted therein, there are considerable limitations in the utility of those markers because there is no marker that can accurately assess both the undifferentiated integrity and differentiated state of stem cells. For example, Oct-4 is a very well validated marker of ES cell pluripotency. However, it is a transcript marker and thus analysis of Oct-4 is destructive to cells such that you can not retain the integrity of the cells during the analysis to allow manipulation of the cells after the analysis. Some other markers such as SSEA-1 are again well validated for use to detect the pluripotency of ES cells, but the kinetics of down-regulation of SSEA-1 after differentiation are non-ideal (i.e. not fast enough down-regulation) for the use in isolation to detect the

differentiated state. Instead, SSEA-1 is generally better for use in combination with other stem cell markers, such as 5T4 as reflected in the above discussion regarding Figure 18.

Thus the above language quoted from page 2, lines 28-29 of the instant application relates to a lack, before the instant disclosure, of a marker that can assess both an undifferentiated state while maintaining cell integrity and a differentiated state in the cells. The instant disclosure regarding 5T4 remedies the previous deficiency in the field.

For instance, Example 1 of the instant application shows that 5T4 antigen is not detected on the surface of undifferentiated mES cells and that 5T4 expression is upregulated following removal of LIF. It is well known in the art that LIF is required to maintain the pluripotency of mES cells and that removal of LIF results in the differentiation of mES cells. This correlation has also been made with the upregulation of 5T4 following removal of LIF and with the differentiation of ES cells by detecting several well-known differentiation markers (Fig 13a) and a decrease in the ES cell-specific Forssman antigen.

Similarly, Example 3 of the instant application shows that human 5T4 expression is negatively associated with pluripotent human ES cells that express the pluripotent marker OCT-4. Moreover, upregulation of cell surface 5T4 expression on the differentiating cell population correlates with loss of the pluripotent marker OCT-4.

Furthermore, and contrary to the conclusion that undue experimentation is needed because "an artisan would have to do further experimentation to determining if cells identified and isolated based of its lack of 5T4 antigen truly can develop into any tissue/cell type" (see last two sentences of paragraph bridging pages 9 and 10), Applicants point out that ES cells are known to exhibit the potential to differentiate into cells of all three germ layers. Figure 14b shows that following spontaneous differentiation of mES cells in culture, 5T4 is expressed on all three primary germ layers derived from the stem cell (endoderm, mesoderm and ectoderm as confirmed using well known germ-layer lineage-specific transcripts from representative cell types). See also Example 1 and page 58, lines 20-27.

Therefore, and contrary to the conclusion of the need for undue experimentation, the presence of 5T4 on cells of all three primary germ layers indicates that differentiation into all

tissue/cell types is expected, and any experimentation to confirm the expectation would be no more than routine and repetitive. As noted above, such experimentation is the opposite of undue.

As for allegation 3), Applicants respectfully submit that the conclusory nature of the statement indicates a failure to apply all the factors set out in *In re Wands* (and listed in the instant statement of the rejection in the paragraph bridging pages 6 and 7). The apparent view being expressed is that the claims are enabled only with respect to subject matter for which guidance is provided. This is clearly not the standard.

Instead, the standard is whether undue experimentation is needed to practice the claimed invention. The fact that some, or even extensive, experimentation may be needed does not necessarily reflect a need for undue experimentation. But allegation 3) includes the conclusion that undue experimentation is needed merely because "[a]n artisan would have to determine if ... other types of stems cells express 5T4 antigen and if its expression pattern is evidence of differentiation status or pluripotency."

Applicants respectfully submit that the above quoted sentence attempts to transform additional experimentation that is merely repetitive and routine, based on the guidance already provided in the instant application and known to the skilled person, into undue experimentation simply because the additional experimentation is not already present in the instant disclosure. This view is clearly in opposition to the applicable *Wands* standard.

Moreover, ES cells have been developed in other species beyond mouse and humans. For example in other primates, such as monkey (see Thomson et al., PNAS. 1995, 92:7844-7848), and in chicken and pig (see Wheeler MB et al., Reprod Fertil Dev. 1994, 6(5):563-8). Therefore, the present state of the art already includes guidance on obtaining ES cells from other species. These additional cells may be tested and used as disclosed in the instant application.

Additionally, the above quoted sentence appears to doubt, without providing an objective basis for the doubt, that the claimed invention is not applicable, or cannot be practiced, with other cell types. This is clearly in opposition to the standard in *In re Marzocchi.*

In light of the deficiencies in the instant rejection as presented above, Applicants respectfully submit that no *prima facie* case of non-enablement has been presented. Accordingly, this rejection is misplaced and may be properly withdrawn.

Issue under 35 U.S.C. §112, Second Paragraph

Claims 2 was rejected under 35 U.S.C. §112, second paragraph as allegedly indefinite for recitation of "low level", which is alleged in the statement of the rejection as rendering the claim indefinite.

Claim 2 has been revised to provide additional context for the inherent meaning and scope of the claimed subject matter. A skilled person in the field would understand the phrase "low or negligible level of 5T4 antigen expression" to be broad, but not indefinite. As set forth by MPEP 2173.04 and the case decisions cited therein, breadth is not indefiniteness.

Applicants respectfully submit that the instant rejection has been rendered moot and may be properly withdrawn.

Issues under 35 U.S.C. §102

Claims 1, 3, 5, 6, 8, and 14 were rejected under 35 U.S.C. §102(b) as allegedly anticipated by Southall et al. Applicants have carefully reviewed the statement of the rejection and respectfully traverse because no *prima facie* case of anticipation has been presented.

As an initial matter, the inclusion of claims 5 and 6 in the instant rejection appears to be in error. Claims 5 and 6 both depend from claim 2 and as such include the feature of claim 2 regarding low expression as indicative of undifferentiated or pluripotent stem cells. This feature is not found in the Southall et al. document, and is the opposite of that disclosure as discussed below.

It is well settled that a *prima facie* case of anticipation requires that all claim features be disclosed in a single reference (see MPEP 2131 and the cases cited therein). Accordingly, no anticipation of claims 5 and 6 is present or possible.

With respect to claims 1, 3, 8 and 14, Southall et al. report the use of the use of an antibody against 5T4 to detect its expression in various cells of normal tissue and neoplastic

cells. But there is no teaching or suggestion that expression of 5T4 may be detected in relation to the differentiation status of stem cells.

The statement of the rejection indicates that "stem cell" as recited in the claims is interpreted as encompassing "most carcinomas". But this does not alter the fact that Southall et al. do not teach or describe detection of 5T4 expression as an indicator of differentiation status in "stem cells," even if the term is construed to encompass "most carcinomas". Southall et al.'s detection of 5T4 expression in cells of normal tissue and neoplastic cells does not relate to differentiation status because 1) the apparent interpretation of "stem cells" excludes cells of normal tissue; 2) cells of normal tissue are differentiated, or relatively more differentiated, than neoplastic cells; and 3) detection of the presence of 5T4 expression in neoplastic cells, or carcinomas, provides no information on differentiation status because, as noted in the statement of the rejection, "most carcinomas are characteristically clonal and undifferentiated".

Therefore, Southall et al.'s disclosure is deficient and cannot relate to detecting 5T4 expression as an indicator of differentiation status because merely detecting the presence of 5T4 expression in neoplastic cells or carcinomas provides no information regarding the differentiation status of such cells. Additionally, the "characteristically undifferentiated" nature of most carcinomas as asserted in the statement of the rejection indicates that as presented by Southall et al., the 5T4 positive status in neoplastic cells is indicative of less differentiation. This is the exact opposite of the situation with stem cells in the instant application and rejected claims.

The deficiency in Southall et al.'s disclosure leaves it unable to meet all the features of claims 1, 3, 8 and 14 as presented above. Accordingly, no anticipation of these claims is present or possible.

Moreover, and should this rejection be maintained despite the above, Applicants point out that the identification of a marker on a cancer cell as disclosed by Southall et al. does not include or suggest the appearance of the same marker antigen on a stem cell because cancer cells are different from stem cells. Cancer cells are also not necessarily characteristically clonal or undifferentiated.

By way of background, stem cells can undergo multi-lineage differentiation, being able to differentiate into multiple cell types, and in the case of pluripotent stem cells, into essentially all cell types found in the adult organism. Cancer cells, while they may sometimes appear undifferentiated, do not have the potential to differentiate into multiple cell types. Rather, they are limited to lineage-restricted differentiation. This includes the example of dermoid cancer offered in the Office Action mailed May 30, 2006. Dermoid cancer cells develop into hair and bone tissue but not other cell types. Additionally, not all cancer cells are clonal or undifferentiated. One such example is evident in the case of glioma.

Additionally, and assumed only for the sake of argument, if cancer cells are clonal and undifferentiated (with which Applicants strenuously disagree), the instant application is based in part on the discovery that 5T4 is not expressed in undifferentiated pluripotent stem cells while 5T4 expression is well known to be upregulated in some cancers. Indeed, 5T4 expression is used by Southall et al. to detect "characteristically undifferentiated" cancer cells as discussed above. This is clearly the opposite of the subject matter of the claims.

In light of the foregoing, Applicants respectfully submit that Southall et al. cannot anticipate the instantly rejected claims. Therefore, no *prima facie* case of anticipation has been presented, and this rejection may be properly withdrawn.

Claims 9 and 10 were rejected under 35 U.S.C. §102(b) as allegedly anticipated by Boyle et al. Applicants have carefully reviewed the statement of the rejection and respectfully traverse because no *prima facie* case of anticipation has been presented.

Claims 9 and 10 feature the separation of undifferentiated and differentiated mammalian stem cells "from a mixture of differentiated and undifferentiated stem cells". Boyle et al., however, only report FACS analysis of 5T4 stained human x mouse hybrid cells, which contained translocated fragments of human chromosome 6 (see page 456, left column). These hybrid cells were all previously described, discrete cell lines that are not "a mixture of differentiated and undifferentiated stem cells" as featured in claims 9 and 10 (see page 455, right column).

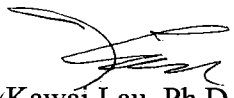
As explained above, it is well settled that a *prima facie* case of anticipation requires that all claim features be disclosed in a single reference. In light of Boyle et al.'s lack of "a mixture of differentiated and undifferentiated stem cells", no anticipation of claims 9 or 10 is present or possible. Accordingly, no *prima facie* case of anticipation has been presented, and this rejection may be properly withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 858-350-6100.

Respectfully submitted,



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